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[54] Title of the Invention: TEST STRIP

[TITLE OF THE INVENTION]

TEST STRIP

[ABSTRACT]

[OBJECT] To provide a test strip for measuring a particular component in a specimen, such as blood sugar, which makes it possible to significantly shorten the time required for the spreading of a specimen such as blood and has very high measurement accuracy.

[MEANS FOR THE ACHIEVEMENT OF THE OBJECT]

A test strip is composed of anisotropic porous membranes stacked one over the other as a first layer and a second layer, respectively. The first layer has a greater average pore size at a surface thereof on a specimen-supplying side than at a surface thereof which is in contact with the second layer. The second layer has a smaller average pore size at a surface thereof, which is in contact with the first layer, than at a surface thereof on a side where a particular component in the specimen is measured.

[CLAIMS]

[Claim 1] A test strip composed of a porous first layer and a second layer stacked one over the other, said first layer carrying a reagent system that can react with a particular component in a specimen, said second layer having a function to filter off suspended matter in said specimen, said test strip being adapted to measure at said second layer said particular component in said specimen by supplying said specimen from said first layer, characterized in that:

said first layer is an anisotropic porous membrane, and has a greater average pore size at a surface thereof on a specimen-supplying side than at a surface thereof which is in contact with said second layer, and

said second layer is an anisotropic porous membrane, and has a smaller average pore size at a surface thereof, which is in contact with said first layer, than at a surface thereof on a side where said particular component in said specimen is measured.

[Claim 2] The test strip according to claim 1, wherein said porous membrane as said first layer has an average pore size of from 3 to 10 $\mu$m, a membrane thickness of from 50 to 200 $\mu$m and a porosity of from 50 to 95%, and a ratio of an average pore size of said surface on said

specimen-supplying side to an average pore size of said surface which is in contact with said second layer is 2.0 or greater.

[Claim 3] The test strip according to claim 1, wherein said porous membrane as said second layer has an average pore size of from 0.1 to 2 $\mu$m, a membrane thickness of from 50 to 200 $\mu$m and a porosity of from 50 to 90%, and a ratio of an average pore size of said surface on the side, where said particular component in said specimen is measured, to an average pore size of said surface, which is in contact with said first layer, is 1.5 or greater.

[Claim 4] The test strip according to any one of claims 1 to 3, wherein said first layer and said second layer are composed of a polyethersulfone.

[Claim 5] The test strip according to any one of claims 1 to 4, wherein said specimen is blood, and said filtered-off matter is primarily composed of blood cells comprising red blood cells.

[Claim 6] The test strip according to any one of claims 1 to 5, wherein said specimen is blood, and said specific component is glucose.

[Claim 7] The test strip according to any one of claims 1 to 6, wherein for hydrophilization, a hydrophilizing agent is contained in said first layer and/or said second

layer.

[Claim 8] The test strip according to any one of claims 1 to 7, wherein said reagent system which can react with said particular component in said specimen comprises at least one of a glucose-oxidase (GOD)-like enzyme, a peroxidase (POD)-like enzyme, an ascorbate-oxidase-like enzyme, an alcohol-oxidase-like enzyme and a cholesterol-oxidase-like enzyme, and at least one of 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine.

[DETAILED DESCRIPTION OF THE INVENTION]

[0001]

[FIELD OF THE INVENTION] This invention relates to a test strip useful with an instrument for measuring the amount of a target component in a specimen, for example, such as a blood sugar measuring instrument (blood component measuring instrument) or in a component-measuring chip to be connected to the instrument.

[0002]

[PRIOR ART] Blood sugar measuring instruments (blood component measuring instruments) are known to perform the measurement of blood sugar levels. These blood sugar measuring instruments each optically measures the degree of a color development on a test strip, which develops a

color in proportion to the level of glucose in blood, (performs a color measurement) to quantitate the blood sugar level. In such a conventional blood sugar measuring instrument, the measurement of a color on a test strip is performed by irradiating light onto the test strip in a light measuring unit, which is equipped with a light-emitting element and a light-receiving element, and measuring the intensity of light reflected from the test strip. Such a blood sugar measuring instrument, however, requires to perform operations such that, after blood (specimen) is supplied to a test paper and is allowed spread over the test strip, the test strip is inserted into a space maintained under light-shielded conditions and a measurement of blood sugar level is then started. Accordingly, the blood sugar measuring instrument is accompanied by a drawback that it is inferior in operability, and moreover, involves a potential problem in that the time from the supply of blood to the test strip until the measurement of its color may not be constant to develop a measurement error. There is, hence, an outstanding desire for the development of an automated blood sugar measuring instrument which permits a series of operations, ranging from the supply and spreading of a specimen to and over a test strip to its measurement, in

a continuous and automated manner.

[0003] Further, conventional test strips are each of the construction that a reagent system is carried on a single sheet of base material composed of a porous material that can absorb a specimen. As the diameters of pores in the sheet of base material are as small as 0.5 $\mu$m or so, this test strip involves a problem in that its water permeability, in other words, its ability to permit spreading (of a specimen) is low and hence, the spreading of the specimen takes time. It is disadvantageous especially for the automated blood sugar measuring instrument that the time required for the spreading of a specimen is long as mentioned above.

[0004] As means for resolving such problems, Japanese Patent Laid-open No. Hei 11-183474 discloses: (1) a test strip composed of a first porous layer, which carries thereon a reagent system that reacts with a particular component in a specimen to develop a color, and a second porous layer, which has a function to filter off solid matter in the specimen, stacked one over the other such that the test strip is used by supplying the specimen from the side of the first layer, (2) the test strip as described above under (1), in which both of the first layer and the second layer have hydrophilicity, (3) the

test strip as described above under (1) or (2), in which the diameters of pores in the first layer are from 8 to 50 $\mu$m, (4) the test strip as described above under any one of (1) to (3), in which the diameters of pores in the second layer are not greater than 5 $\mu$m, and (5) the test strip as described above under any one of (1) to (4), in which the specimen is blood and the solid matter is primarily composed of blood cells including red blood cells.

[0005] The use of a test strip composed of separate layers, a first layer and a second layer, as described above is described to resolve the above-mentioned problems. However, the use of such a test strip is accompanied by problems as will be described hereinafter. A porous membrane as the first layer is required to have pores of a size sufficient to also permit permeation of blood cells such that blood (specimen) is allowed to promptly spread throughout the membrane and a component to be measured is caused to react with a reagent system carried on surfaces of a porous structure in the membrane to develop a color. It may, therefore, be contemplated to make the pore size greater because a greater pore size makes it possible to achieve faster spreading of blood. An unduly large pore size, however, provides the porous

material with a smaller surface area, leading to a reduction in the amount of a reagent system which can be carried on the surfaces. Especially when the concentration of a target substance in a specimen is high, the amount of the reagent system becomes insufficient compared with the amount of the target substance, thereby failing to perform any accurate measurement.

[0006] The porous membrane as the second layer is required to permit the prompt spreading of a plasma component, which has reacted with the reagent system, over a measurement surface while filtering off blood cells. The smaller the pore size, the more effective for the filtering-off and elimination of blood cells. An excessively small pore size, however, leads to slower spreading of the plasma component. There is a method that makes the pore size large on an inlet side and small on an outlet side to eliminate blood cells while maintaining a spreading rate. When the elimination of blood cells is conducted immediately upstream of a measurement surface, however, hemoglobin as a blood cell component is visible through the porous structure to affect the accuracy of the measurement.

[0007]

[OBJECTS TO BE ACHIEVED] Objects of the present invention

is to provide a test strip for measuring a specific component in a specimen, which can significantly shorten the time required for the spreading of the specimen and has a very high measurement accuracy.

[0008]

[MEANS FOR ACHIEVING THE OBJECTS] These objects can be achieved by the present invention as will be described below.

(1) This invention provides a test strip composed of a porous first layer and a second layer stacked one over the other, the first layer carrying a reagent system that can react with a particular component in a specimen, the second layer having a function to filter off suspended matter in the specimen, the test strip being adapted to measure at the second layer the particular component in the specimen by supplying the specimen from the first layer, characterized in that the first layer is an anisotropic porous membrane, and has a greater average pore size at a surface thereof on a specimen-supplying side than at a surface thereof which is in contact with the second layer, and the second layer is an anisotropic porous membrane, and has a smaller average pore size at a surface thereof, which is in contact with the first layer, than at a surface thereof on a side where the particular

component in the specimen is measured.

[0009] (2) This invention also provides the test strip as described above under (1), wherein the porous membrane as the first layer has an average pore size of from 3 to 10 $\mu$m, a membrane thickness of from 50 to 200 $\mu$m and a porosity of from 50 to 95%, and a ratio of an average pore size of the surface on the specimen-supplying side to an average pore size of the surface which is in contact with the second layer is 2.0 or greater.

(3) This invention also provides the test strip as described above under (1), wherein the porous membrane as the second layer has an average pore size of from 0.1 to 2 $\mu$m, a membrane thickness of from 50 to 200 $\mu$m and a porosity of from 50 to 90%, and a ratio of an average pore size of the surface on the side, where the particular component in the specimen is measured, to an average pore size of the surface, which is in contact with the first layer, is 1.5 or greater.

[0010] (4) This invention also provides the test strip as described above under any one of (1) to (3), wherein the first layer and the second layer are composed of a polyethersulfone.

(5) This invention further provides the test strip as described above under any one of (1) to (4), wherein

the specimen is blood, and the filtered-off matter is primarily composed of blood cells including red blood cells.

(6) This invention further provides the test strip as described above under any one of (1) to (5), wherein the specimen is blood, and the specific component is glucose.

(7) This invention still further provides the test strip as described above under any one of (1) to (6), wherein for hydrophilization, a hydrophilizing agent is contained in the first layer and/or the second layer.

[0011] (8) This invention still further provides the test strip as described above under any one of (1) to (7), wherein the reagent system which can react with the particular component in the specimen includes at least one of a glucose-oxidase (GOD)-like enzyme, a peroxidase (POD)-like enzyme, an ascorbate-oxidase-like enzyme, an alcohol-oxidase-like enzyme and a cholesterol-oxidase-like enzyme, and at least one of 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine.

[0012]

[MODES FOR CARRYING OUT THE INVENTION] The test strip according to the present invention is composed of the first layer and the second layer stacked one over the

other. The first layer serves to supply the specimen from its open surface, and further, to react the specific component in the specimen with the reagent system that can react with the specific component, while the second layer serves to filter off the suspended matter in the specimen, and further, to measure at its open surface the specific component in the specimen. The first layer and second layer are principally equipped with the following constructions.

[0013] The first layer is an anisotropic porous membrane, and has a greater average pore size at the surface thereof on the specimen-supplying side than at the surface thereof which is in contact with the second layer. The first layer is needed to accelerate the permeation and spreading of the specimen, to carry the reagent system in an amount as much as needed, and to provide a place where the reaction of the specimen with the reagent system takes place. The accelerate of the permeation of the specimen into the first layer can be achieved by making the pore size greater at the surface of the porous membrane making up the first layer, the surface being on the specimen-supplying side. However, an enlargement of the pore size throughout the membrane leads to a reduction in the surface area with which the specimen can

be brought into contact, and therefore, to a reduction in the amount of the reagent system which can be carried. The adoption of an anisotropic structure that the pore size is enlarged on the side where the specimen is supplied and the pore size is reduced on the other side, therefore, has made it possible to accelerate the permeation and spreading of the specimen, to carry the reagent system in an amount as much as needed, and also to provide a place where the reaction of the specimen with the reagent system takes place. As a consequence, a prompt and accurate measurement has become feasible.

[0014] As a specific structure of the anisotropic porous membrane as the first layer, its average pore size may be from 3 to 10 $\mu$m, desirably from 3 to 7 $\mu$m, more desirably from 3 to 5 $\mu$m, and the ratio of the average pore size of the surface, onto which the specimen is supplied, to the average pore size of the surface, which is brought into contact with the second layer, may be 2.0 or greater, desirably 3.0 or greater, more desirably 4.0 or greater.

[0015] The membrane thickness of the first layer may be from 50 to 200 $\mu$m, desirably from 70 to 180 $\mu$m, more desirably from 80 to 150 $\mu$m, although no particular limitation is imposed thereon. Because, a membrane

thickness smaller than 50 $\mu$m results in insufficient membrane strength, while a membrane thickness greater than 200 $\mu$m requires an excessively long time for the spreading of a specimen.

[0016] The porosity of the first layer may be from 50 to 95%, desirably from 60 to 90%, more desirably from 70 to 88%, although no particular limitation is imposed thereon. A porosity lower than 50% leads to a failure in absorbing and spreading a specimen in an amount as much as needed, while a porosity higher than 95% leads to insufficient membrane strength.

[0017] Examples of a polymer usable as a membrane material for the first layer include nitrocellulose, polyvinyl difluoride, cellulose acetate, polysulfones, polyethersulfones, and polyethylene. Especially when a reagent system is carried for use in measuring a blood sugar level, a polyethersulfone can be suitably used as the activity of the reagent system is deteriorated least with time.

[0018] Examples of the reagent system which can be carried on the first layer include enzyme preparations of glucose-oxidase (GOD)-like enzymes, peroxidase (POD)-like enzymes, ascorbate-oxidase-like enzymes, alcohol-oxidase-like enzymes, and cholesterol-oxidase-like enzymes; and

color developers such as 4-aminoantipyrine and N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine. It is to be noted that these reagents can be carried either singly or in combination on the test strip. The carrying method can include, but is not limited to, a method which includes dissolving such a reagent system in a buffer, such as phosphate buffer, or water, impregnating the first layer with the thus-prepared solution, and then drying the impregnated first layer.

[0019] The second layer is an anisotropic porous membrane, and has a smaller average pore size at the surface which is in contact with the first layer than at the surface on the side where the specific component in the specimen is measured. The second layer is required to filter off suspended matter such as blood cells and also to cause prompt spreading of the specific component such as a plasma component, which has reacted with the reagent system, to the open surface. The filtering off and elimination of suspended matter such as blood cells can be achieved by making the pore size smaller. However, an excessively small pore size leads to slow spreading of the specific component such as the plasma component. There is also a method which, while maintaining a spreading rate, eliminates suspended matter such as blood

cells by making the pore size greater at the surface which is in contact with the first layer and smaller at the surface on the side where the measurement is performed. The filtering off and elimination of the suspended matter is, however, conducted in the proximity of the surface on the side where the specific component is measured. Accordingly, hemoglobin as a blood cell component is visible through the porous structure to affect the accuracy of the measurement.

[0020] It is, therefore, possible to exactly perform the filtering off and elimination of suspended matter such as blood cells and also to improve the spreading rate of a specific component such as a plasma component to the surface on the side, where the measurement is performed and the accuracy of measurement, by forming an anisotropic structure that the pore size is made smaller at the surface, which is in contact with the first layer, and greater at the opposite surface.

[0021] As a specific structure of the anisotropic porous membrane as the second layer, its average pore size may be from 0.1 to 2 $\mu$m, desirably from 0.3 to 1.6 $\mu$m, more desirably from 0.5 to 1.3 $\mu$m, and the ratio of the average pore size of the surface on the side, where the specific component in the specimen is measured, to the

average pore size of the surface, which is in contact with the first layer, may be 1.5 or greater, desirably 2.0 or greater, more desirably 2.3 or greater.

[0022] The thickness of the second layer may be from 50 to 200 $\mu$m, desirably from 70 to 180 $\mu$m, more desirably from 80 to 150 $\mu$m, although no particular limitation is imposed thereon. Because, a membrane thickness smaller than 50 $\mu$m results in insufficient membrane strength, while a membrane thickness greater than 200 $\mu$m requires an excessively long time for the spreading of a specimen.

[0023] The porosity of the second layer may be from 50 to 95%, desirably from 70 to 90%, more desirably from 75 to 85%, although no particular limitation is imposed thereon. Because a porosity lower than 50% leads to a failure in absorbing and spreading a specimen in an amount as much as needed, while a porosity higher than 95% leads to insufficient membrane strength.

[0024] Examples of a polymer usable as a membrane material for the second layer include nitrocellulose, polyvinyl difluoride, cellulose acetate, polysulfones, polyethersulfones, and polyethylene. Especially when a reagent system is carried for use in measuring a blood sugar level, a polyethersulfone can be suitably used as the activity of the reagent system is deteriorated least

with time.

[0025] Concerning the first layer and second layer, it is desired to produce it by impregnating it with an aqueous solution with a reagent system dissolved therein, and for the prompt supply and spreading of a specimen, it is also desired to incorporate a hydrophilizing agent, to form the anisotropic porous membrane with a material having hydrophilicity, or to apply hydrophilization processing. Examples of the hydrophilizing agent include surfactants such as "TRITON X-100", water-soluble silicones, hydroxypropylcellulose, polyethylene glycol, and polypropylene glycol. Examples of the hydrophilization processing include plasma processing, glow discharge, corona discharge, and ultraviolet exposure.

[0026] No particular limitation is imposed on the production method of the first layer and the second layer, and they can be obtained by wet film-forming, dry film-forming, melt film-forming, or the like. As a specific example, a method can be mentioned which includes coating a polymer solution as a membrane material out into a film-like form and removing the solvent component to dry the membrane material.

[0027] No particular limitation is imposed either on the stacking method of the first layer and second layer. As

an illustrative method, the first and second layers may be simply stacked one over the other and may then be fixed together along the circumferences thereof, or the first and second layers may be bonded together with an adhesive or may be fusion-bonded with each other.

[0028] It is to be noted that the test strip according to the present invention is used by inserting it into a chip which can be detachably mounted on an instrument for measuring a particular component in a specimen or into a measuring instrument itself. Examples of the measuring instrument include instruments for quantitatively or qualitatively measuring the sugar, cholesterol, fat and the like in blood or the sugar, proteins, occult blood and the like in urine.

[0029]

[Examples] Specific examples of the present invention will hereinafter be described. As Examples 1 to 7, Comparative Examples 1 to 2 and Comparative Examples 6 to 8, anisotropic porous membranes for use as the first and second layers of the examples and comparative examples were formed under the conditions to be described hereinafter. (In each of the examples and comparative examples), the film-forming solution shown in Table 1 was firstly supplied in the form of a line on a substrate

(glass plate) by a 50 mL syringe, and was then spread over the glass plate by an applicator with a 125 $\mu$m gap. The glass plate with the film-forming solution spread thereon was immersed in a solidification medium composed of a 70% aqueous solution of N-methyl-2-pyrrolidone (NMP) prepared at the temperature shown in Table 2, and the polymer component as the membrane material was caused to deposit. Subsequently, the solvent component and the water-soluble additive component were extracted out in a water bath, followed by drying in an oven of 40°C to obtain a porous membrane.

[0030] In Comparative Examples 3 to 5 and Comparative Examples 9 to 10, commercially-available nitrated polyethersulfone membranes and polyethersulfone membranes (both, products of Pall Gelman Sciences Inc.) were used, respectively.

[0031]

[Table 1]

Table 1 Compositions of Film-forming Solutions

| Film-forming solution for the first layer | |
|---|---|
| Polyethersulfone ("SUMIKAEXEL 7300P", product of Sumitomo Chemical Co., Ltd.) | 10 wt% |
| Polyvinylpyrrolidone ("BASF POLYVINYLPYRROLIDONE K-90", product of BASF Corporation) | 5 wt% |
| N-Methyl-2-pyrrolidone (product of BASF Corporation) | 85 wt% |
| Film-forming solution for the second layer | |
| Polyethersulfone ("SUMIKAEXEL 7300P", product of Sumitomo Chemical Co., Ltd.) | 15 wt% |
| Polyvinylpyrrolidone ("BASF POLYVINYLPYRROLIDONE K-90", product of BASF Corporation) | 7.5 wt% |
| N-Methyl-2-pyrrolidone (product of BASF Corporation) | 77.5 wt% |

[0032]

[Table 2]

Table 2 Film-forming Solidification Temperature

| | | Temperature of solidification medium (°C) |
|---|---|---|
| First layer | Comparative Example 1 | 25 |
| | Example 1 | 30 |
| | Example 2 | 35 |
| | Example 3 | 40 |
| | Comparative Example 2 | 45 |
| Second layer | Comparative Example 6 | 25 |
| | Example 4 | 30 |
| | Example 5 | 35 |
| | Example 6 | 40 |
| | Example 7 | 45 |
| | Comparative Example 7 | 50 |
| | Comparative Example 8 | 55 |

[0033] Various physical properties of the anisotropic porous membranes of the respective examples and comparative examples are shown in Table 3 and Table 4. The average pore size of the porous membrane of each example or comparative example was measured by capillary flow porometry in accordance with ASTM F316-86. As a measuring instrument, "PALM POROSIMETER" (manufactured by Porous Materials Inc.) was used. Concerning the average surface pore size of the porous membrane of each example or comparative example, an image taken by a scanning electron microscope ("JSM-840", manufactured by JEOL, Ltd.) was analyzed by an image analyzer ("IP-1000PC", manufactured by Asahi Kasei Corporation) such that the diameters of pores in a field of vision were calculated as circle equivalent diameters in terms of area and their mean was recorded as the average surface pore size. Therefore, a correlation is not necessarily established between the average pore size and average surface pore size of each porous membrane. The thickness of each membrane was measured by a micrometer (manufactured by MITUTOYO CORPORATION). Each porosity was measured by the weight method. Details of the individual porous membranes were as follows.

[0034] [First layer]

Material: Polyethersulfone

Thickness: 130 ± 5 μm

Porosity: 82 ± 3%

Coated reagent system: GOD, POD and 4-aminoantipyrine, N-ethyl-N-(2-hydroxy-3-sulfopropyl)-m-toluidine (TOOS), "Triton X-100"

[0035] [Second layer]

Material: Polyethersulfone

Thickness: 100 ± 5 μm

Porosity: 84 ± 3%

Coated reagent: "Triton X-100"

[0036]

[Table 3]

Table 3

| First layer | Average pore size (μm) | Average surface pore size (μm) | | Ratio of large pore size/small pore size |
|---|---|---|---|---|
| | | Open surface (small pore-size surface) | Substrate-side surface (large pore-size surface) | |
| Comparative Example 1 Anisotropic membrane | 1.0 | 0.2 | 2.0 | 8.9 |
| Example 1 Anisotropic membrane | 3.1 | 0.2 | 2.4 | 10.6 |
| Example 2 Anisotropic membrane | 5.0 | 0.2 | 4.7 | 21.5 |
| Example 3 Anisotropic membrane | 10.0 | 0.3 | 5.5 | 21.1 |
| Comparative Example 2 Anisotropic membrane | 15.0 | 0.3 | 6.3 | 20.4 |
| Comparative Example 3 Isotropic membrane | 1.0 | 0.8 | 1.0 | 1.2 |
| Comparative Example 4 Isotropic membrane | 2.6 | 2.4 | 2.5 | 1.1 |
| Comparative Example 5 Isotropic membrane | 5.0 | 4.6 | 4.8 | 1.0 |

[0037]

[Table 4]

Table 4

| Second layer | Average pore size (μm) | Average surface pore size (μm) | | Ratio of large pore size/small pore size |
|---|---|---|---|---|
| | | Open surface (small pore-size surface) | Substrate-side surface (large pore-size surface) | |
| Comparative Example 6 Anisotropic membrane | 0.05 | 0.1 | 0.2 | 3.6 |
| Example 4 Anisotropic membrane | 0.10 | 0.1 | 0.3 | 2.4 |
| Example 5 Anisotropic membrane | 0.5 | 0.2 | 1.4 | 7.8 |
| Example 6 Anisotropic membrane | 1.0 | 0.2 | 2.0 | 8.9 |
| Example 7 Anisotropic membrane | 2.0 | 0.2 | 1.9 | 8.5 |
| Comparative Example 7 Anisotropic membrane | 3.1 | 0.2 | 2.4 | 10.6 |
| Comparative Example 8 Anisotropic membrane | 5.0 | 0.2 | 4.7 | 21.5 |
| Comparative Example 9 Isotropic membrane | 0.22 | 0.5 | 0.6 | 1.2 |
| Comparative Example 10 Isotropic membrane | 0.45 | 1.0 | 1.3 | 1.3 |

[0038] (Test 1) Using the porous membranes of the first layers of the examples and comparative examples, the following experiment was conducted. Each porous membrane to be evaluated was fixed on a sample holder of a spectrophotometer ("UV-2400(PC)S", manufactured by SHIMADZU CORPORATION) to permit the measurement of a reflection absorbance at the surface of the membrane. Human blood (5 μL) was added to the surface, which was opposite to the measurement surface, by a micropipette (manufactured by Eppendorf Co., Ltd.), and changes in reflection absorbance on the opposite surface were

measured with time. With respect to each anisotropic membrane, the addition of the blood was conducted in two ways, one being to the surface of greater pore size, and the other to the surface of smaller pore size. The period from a time point at which the rate of a change per second in reflectance exceeded 1% of the final change until a time point at which the rate of a change per second in reflectance fell short of 1% of the final change was used as a soak-through time ($\Delta t$). Measurement conditions were as described below. The results are shown in Table 5.

[0039] Measurement conditions (changes with time)

Measurement value: reflectance

Wavelength: 610 nm

Slit width: 2.0 nm

Timing mode: auto

Measurement time: 90 sec

Sampling pitch: 0.1 sec

Cell number: 1

Data number: 901

[0040]

[Table 5]

Table 5

| First layer | Test 1 Soak-through time Δt | | Test 2 |
|---|---|---|---|
| | Added from the large pore-size surface (sec) | Added from the small pore-size surface (sec) | Carried amount of reagent system (mg/cm$^2$) |
| Comparative Example 1 Anisotropic membrane | 8.3 | 5.7 | 1.1 |
| Example 1 Anisotropic membrane | 2.6 | 4.6 | 1.0 |
| Example 2 Anisotropic membrane | 1.8 | 3.5 | 1.0 |
| Example 3 Anisotropic membrane | 1.4 | 3.1 | 0.9 |
| Comparative Example 2 Anisotropic membrane | 0.8 | 2.6 | 0.7 |
| Comparative Example 3 Isotropic membrane | 10.9 | - | 2.2 |
| Comparative Example 4 Isotropic membrane | 4.4 | - | 1.5 |
| Comparative Example 5 Isotropic membrane | 2.9 | - | 0.6 |

[0041] (Test 2) With respect to the porous membrane of the first layer of each of the examples and comparative examples, the carried amount of the reagent system was measured as will be described below. The weight of the porous membrane was precisely measured before the coating of the reagent system thereon, and was subtracted from the weight of the porous membrane after the coating to calculate the carried amount of the reagent system. The results are shown in Table 5.

[0042] (Test 3) Using the porous membranes of the second layers of the examples and comparative examples, the

following experiment was conducted. Each porous membrane to be evaluated was fixed on a sample holder of a spectrophotometer ("UV-2400(PC)S", manufactured by SHIMADZU CORPORATION) to permit the measurement of a reflection absorbance at the surface of the membrane. Human blood (5 $\mu$L) was added to the surface, which was opposite to the measurement surface, by a micropipette (manufactured by Eppendorf Co., Ltd.). A soak-through time ($\Delta$t) was measured as in Test 1, and a reflective absorption spectrum of reflection absorbance on the opposite surface was also measured. Measurement conditions for the soak-through time ($\Delta$t) were the same as in Test 1. Compared with a spectrum measured when plasma was added, it was determined whether or not the measurement was affected by hemoglobin. With respect to each anisotropic membrane, the addition of the blood was conducted in two ways, one being to the surface of greater pore size, and the other to the surface of smaller pore size. The results are shown in Table 6.

[0043] Measurement conditions (reflective absorption spectrum)

Measurement value: reflectance

Wavelength range (nm): 700 (start) 500 (end)

Scanning speed: medium

Slit width: 2.0 nm

Sampling pitch: 1.0 nm

[0044]

[Table 6]

Table 6

| Second layer | Test 3 | | | |
|---|---|---|---|---|
| | Soak-through time Δt | | Effect of hemoglobin | |
| | Added from the large pore-size surface (sec) | Added from the small pore-size surface (sec) | Large pore-size surface | Small pore-size surface |
| Comparative Example 6 Anisotropic membrane | 10.2 | 37.7 | Not affected | Not affected |
| Example 4 Anisotropic membrane | 7.2 | 35.2 | Not affected | Not affected |
| Example 5 Anisotropic membrane | 6.8 | 15.9 | Not affected | Not affected |
| Example 6 Anisotropic membrane | 5.7 | 8.3 | Affected | Not affected |
| Example 7 Anisotropic membrane | 5.2 | 5.4 | Affected | Not affected |
| Comparative Example 7 Anisotropic membrane | 4.6 | 2.6 | Affected | Not affected |
| Comparative Example 8 Anisotropic membrane | 3.5 | 1.8 | Affected | Affected |
| Comparative Example 9 Isotropic membrane | 8.7 | - | Affected | - |
| Comparative Example 10 Isotropic membrane | 8.1 | - | Affected | - |

[0045] (Test 4) The following experiment was conducted to stack the porous membranes as the first layers with their corresponding porous membranes as the second layers. Each combination of porous membranes as the first layer and second layer was fixed on a sample holder of a spectrophotometer ("UV-2400(PC)S", manufactured by SHIMADZU CORPORATION) such that the porous membranes were

stacked one over the other to keep them in close contact with each other. Human blood (5 $\mu$L) was added to the inlet side by a micropipette (manufactured by Eppendorf Co., Ltd.), and on the opposite surface, changes with time in reflection absorbance and a reflective absorption spectrum were measured.

[0046] At that time, the porous membrane as the first layer and the porous membrane as the second layer were fixed on the blood-adding side and the measuring side, respectively. Concerning the directions in which the respective porous membranes were fixed, the experiment was conducted in such a way that except for the isotropic membranes, the first layer was fixed with its large pore-size surface being placed as the blood-adding side and the second layer was fixed with its large pore-size surface being placed as the measuring side. The period from a time point at which the rate of a change per second in reflectance exceeded 1% of the final change until a time point at which the rate of a change per second in reflectance fell short of 1% of the final change was used as a soak-through time ($\Delta t$).

[0047] Compared with a spectrum measured when plasma was added, it was determined whether or not the measurement was affected by hemoglobin. Respective measurement

conditions were the same as in Test 1 and Test 3. The results of those measurements are shown in Table 7.

[0048]

[Table 7]

Table 7

| | | | Test 4 | |
|---|---|---|---|---|
| | First layer | Second layer | Soak-through time Δt (sec) | Effect of hemoglobin |
| Comparative Example 11 | Comparative Example 1 | Comparative Example 6 | 15.3 | Not affected |
| Comparative Example 12 | Comparative Example 1 | Example 4 | 15.3 | Not affected |
| Comparative Example 13 | Example 1 | Comparative Example 6 | 11.9 | Not affected |
| Example 8 | Example 1 | Example 4 | 9.1 | Not affected |
| Example 9 | Example 1 | Example 7 | 7.4 | Not affected |
| Example 10 | Example 2 | Example 5 | 7.7 | Not affected |
| Example 11 | Example 2 | Example 6 | 6.5 | Not affected |
| Example 12 | Example 3 | Example 4 | 8.6 | Not affected |
| Example 13 | Example 3 | Example 7 | 6.2 | Not affected |
| Comparative Example 14 | Example 3 | Comparative Example 7 | 6.0 | Affected |
| Comparative Example 15 | Comparative Example 3 | Comparative Example 9 | 18.1 | Not affected |
| Comparative Example 16 | Comparative Example 5 | Comparative Example 10 | 9.8 | Affected |

[0049]

[EFFECTS OF THE INVENTION] The test strip according to the present invention is fast in the spreading rate of a specimen and can shorten the time required for spreading,

and upon colorimetric measurement, assures a sufficient carried amount of a reagent system, can eliminate effects of suspended matter, and permits a measurement with higher accuracy. Described specifically, the present invention can provide a test strip for the measurement of a specific component in a specimen, which can significantly shorten the time required for the spreading of the specimen and is very high in the accuracy of measurement.

[0050] In particular, the present invention is effective as a blood sugar test strip for measuring sugar in blood. It is high in the spreading rate of blood and can shorten the time required for its spreading, and upon measurement of the blood sugar level, assures a sufficient carried amount of a reagent system of an enzyme preparation, a color developer and the like, can eliminate effects of suspended matter such as red blood cells, and can perform the measurement with higher accuracy.

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(54)【発明の名称】　試験紙

(57)【要約】
【課題】血液などの検体の展開に要する時間を飛躍的に短縮することができ、かつ非常に測定精度が高い、血糖などの検体中の特定成分を測定する試験紙を提供する。
【解決手段】異方性の多孔質膜の第１層と第２層を積層してなり、第１層は検体を供給する側の表面の平均孔径が第２層と接触する側の表面の平均孔径よりも大きいものであり、第２層は第１層と接触する側の表面の平均孔径が前記検体中の特定成分を測定する側の表面の平均孔径よりも小さいものである試験紙。

【特許請求の範囲】
【請求項１】検体中の特定成分と反応する試薬を担持する多孔質の第１層と、前記検体中の浮遊物を濾別する機能を有する第２層とを積層してなり、前記第１層から前記検体を供給して前記第２層で前記検体中の特定成分を測定する試験紙において、
前記第１層が異方性の多孔質膜で、前記検体を供給する側の表面の平均孔径が前記第２層と接触する側の表面の平均孔径よりも大きいものであり、
前記第２層が異方性の多孔質膜で、前記第１層と接触する側の表面の平均孔径が前記検体中の特定成分を測定する側の表面の平均孔径よりも小さいことを特徴とする試験紙。
【請求項２】前記第１層の多孔質膜が、平均孔径が３～１０μｍ、膜厚が５０～２００μｍ、及び空孔率が５０～９５％であり、前記検体を供給する側の表面の平均孔径と前記第２層と接触する側の表面の平均孔径との比が２．０以上であることを特徴とする請求項１に記載の試験紙。
【請求項３】前記第２層の多孔質膜が、平均孔径が０．１～２μｍ、膜厚が５０～２００μｍ、及び空孔率が５０～９０％であり、前記検体中の特定成分を測定する側の表面の平均孔径と前記第１層と接触する側の表面の平均孔径との比が１．５以上であることを特徴とする請求項１に記載の試験紙。
【請求項４】前記第一層及び前記第二層がポリエーテルスルホンからなることを特徴とする請求項１乃至３のいずれかに記載の試験紙。
【請求項５】前記検体は血液であり、前記濾別物は主に赤血球を含む血球である請求項１乃至４のいずれかに記載の試験紙。
【請求項６】前記検体は血液であり、前記特定成分はグルコースである請求項１乃至５のいずれかに記載の試験紙。
【請求項７】前記第一層及び／又は前記第二層に親水化のために親水化剤を含む請求項１乃至６のいずれかに記載の試験紙。
【請求項８】前記検体中の特定成分と反応する試薬が、グルコースオキシターゼ（ＧＯＤ）様酵素、ペルオキシターゼ（ＰＯＤ）様酵素、アスコルビン酸オキシダーゼ様酵素、アルコールオキシダーゼ様酵素、及びコレステロールオキシダーゼ様酵素の少なくとも一つ、及び４－アミノアンチピリン、Ｎ－エチル－Ｎ－（２－ヒドロキシ－３－スルホプロピル）－ｍ－トルイジンの少なくとも一つから構成されるものである請求項１乃至７のいずれかに記載の試験紙。
【発明の詳細な説明】
【０００１】
【発明が属する技術分野】本発明は、例えば血糖測定装置（血中成分測定装置）のような検体中の目的成分の量を測定する装置または当該装置に接続する成分測定用チップに用いる試験紙に関するものである。
【０００２】
【従来の技術】血糖値の測定を行う血糖測定装置（血中成分測定装置）が知られている。この血糖測定装置は、血中のブドウ糖量に応じて呈色する試験紙の呈色の度合いを光学的に測定（測色）して血糖値を定量化するものである。このような従来の血糖測定装置では、試験紙の測色は、発光素子および受光素子を備える測光部において、試験紙に光を照射しその反射光の強度を測定することにより行われている。この血糖測定装置では、試験紙に血液（検体）を供給・展開する操作を行った後、その試験紙を遮光状態が確保される空間へ挿入し、血糖値の測定を開始するが、操作性が劣るという欠点があるとともに、試験紙への血液の供給から測色までの時間が一定でなく、それによる測定誤差が生じるという問題がある。そのため、試験紙への供給・展開から測定までの一連の操作を連続的、自動的に行うことが出来る血糖自動測定装置の開発が望まれている。
【０００３】また、従来の試験紙は、検体を吸収可能な多孔質材料で構成された１枚のシート基材に試薬を担持させた構成のものである。この試験紙では、シート基材の細孔の孔径が０．５μｍ程度と小さいため、通水性、すなわち展延性が低く、そのため検体の展開に時間がかかるという問題がある。このように検体の展開に要する時間が長いということは、とくに、前記血糖自動測定装置にとって不利である。
【０００４】また、このような問題点を解決する手段として、（１）検体中の特定成分と反応して呈色する試薬を担持する多孔質の第１の層と、検体中の濾別物を濾別する機能を有する多孔質の第２の層とを積層してなり、前記第１の層側から検体を供給して使用することを特徴とする試験紙、（２）前記第１の層および前記第２の層がそれぞれ親水性を有している上記（１）に記載の試験紙、（３）前記第１の層における細孔の孔径が８～５０μｍである上記（１）または（２）に記載の試験紙、（４）前記第２の層における細孔の孔径が５μｍ 以下である上記（１）ないし（３）のいずれかに記載の試験紙、及び（５）前記検体は血液であり、前記濾別物は主に赤血球を含む血球である上記（１）ないし（４）のいずれかに記載の試験紙が、特開平１１－１８３４７４号で示されている。
【０００５】以上のような第１の層と第２の層に分かれた試験紙を使用することで、前述の問題が解決されるという。しかしながらこのような試験紙を用いた場合にも、以下のような問題点がある。まず、第１層目の多孔質膜は、血球をも透過させ得る大きさの孔を持つことで血液（検体）を迅速に膜内に展開させ、膜内の多孔質構造表面に担持してある試薬と測定成分とを反応させて呈色させることが要求される。したがって、孔径を大きく

することでより早い血液の展開が得られるため、孔径を大きくすればする程良いと考えられる。ところが孔径を大きくしすぎると、多孔質体の表面積が少なくなり、表面に担持される試薬の量が少なくなってしまい、特に検体中の被測定物質の濃度が濃い場合、被測定物質の量に比べて試薬の量が不足してしまい、正確な測定が出来なくなる。

【０００６】また、第２層目の多孔質膜は、血球を濾別しつつ試薬と反応した血漿成分を迅速に測定面へ展開させうることが要求される。血球を濾別・除去するためには孔径を小さくする程効果があるが、孔径を小さくしすぎると血漿成分の展開が遅くなる。また、入り口側の孔径を大きくし、出口側の孔径を小さくすることで展開速度を維持しつつ血球を除去する方法もあるが、血球除去が測定面直前で行われたのでは、血球成分の血色素が多孔質構造を通して透けて見えてしまい、測定精度に影響してしまう。

【０００７】

【発明が解決しようとする課題】本発明の目的は、検体の展開に要する時間を飛躍的に短縮することができ、かつ非常に測定精度が高い、検体中の特定成分測定用試験紙を提供することにある。

【０００８】

【課題を解決するための手段】このような目的は、以下の本発明により達成される。

（１）本発明は、検体中の特定成分と反応する試薬を担持する多孔質の第１層と、前記検体中の浮遊物を濾別する機能を有する第２層とを積層してなり、前記第１層から前記検体を供給して前記第２層で前記検体中の特定成分を測定する試験紙において、前記第１層が異方性の多孔質膜で、前記検体を供給する側の表面の平均孔径が前記第２層と接触する側の表面の平均孔径よりも大きいものであり、前記第２層が異方性の多孔質膜で、前記第１層と接触する側の表面の平均孔径が前記検体中の特定成分を測定する側の表面の平均孔径よりも小さいことを特徴とする試験紙である。

【０００９】（２）本発明は、前記第１層の多孔質膜が、平均孔径が３～１０μｍ、膜厚が５０～２００μｍ、及び空孔率が５０～９５％であり、前記検体を供給する側の表面の平均孔径と前記第２層と接触する側の表面の平均孔径との比が２．０以上であることを特徴とする上記（１）に記載の試験紙である。

（３）本発明は、前記第２層の多孔質膜が、平均孔径が０．１～２μｍ、膜厚が５０～２００μｍ、及び空孔率が５０～９０％であり、前記検体中の特定成分を測定する側の表面の平均孔径と前記第１層と接触する側の表面の平均孔径との比が１．５以上であることを特徴とする上記（１）に記載の試験紙である。

【００１０】（４）本発明は、前記第一層及び前記第二層がポリエーテルスルホンからなることを特徴とする上記（１）乃至（３）のいずれかに記載の試験紙である。

（５）本発明は、前記検体は血液であり、前記濾別物は主に赤血球を含む血球である上記（１）乃至（４）のいずれかに記載の試験紙である。

（６）本発明は、前記検体は血液であり、前記特定成分はグルコースである上記（１）乃至（５）のいずれかに記載の試験紙である。

（７）本発明は、前記第一層及び／又は前記第二層に親水化のために親水化剤を含む上記（１）乃至（６）のいずれかに記載の試験紙である。

【００１１】（８）本発明は、前記検体中の特定成分と反応する試薬が、グルコースオキシターゼ（ＧＯＤ）様酵素、ペルオキシターゼ（ＰＯＤ）様酵素、アスコルビン酸オキシダーゼ様酵素、アルコールオキシダーゼ様酵素、及びコレステロールオキシダーゼ様酵素の少なくとも一つ、及び４－アミノアンチピリン、Ｎ－エチル－Ｎ－（２－ヒドロキシ－３－スルホプロピル）－ｍ－トルイジンの少なくとも一つから構成されるものである上記（１）乃至（７）のいずれかに記載の試験紙である。

【００１２】

【発明の実施の形態】本発明の試験紙は第１層と第２層とを積層してなるものである。第１層は、開放面から検体を供給し、かつ検体中の特定成分と当該反応する試薬を反応させるものであり、第２層は検体中の浮遊物を濾別し、かつ開放面で検体中の特定成分を測定するものである。第１層及び第２層は主として以下の構成を有するものである。

【００１３】第１層は、異方性の多孔質膜で、検体を供給する側の表面の平均孔径が前記第２層と接触する側の表面の平均孔径よりも大きいものである。第１層は、検体の浸み込み及び展開を早くし、必要量の試薬を担持し、かつ検体と試薬が反応する場を提供することが必要とされ、第１層への検体の浸み込みを早くするためには、第１層を構成する多孔質膜の検体が供給される側の面の孔径を大きくすることで解決できる。しかし、膜全体にわたって孔径を大きくすると、検体が接触する表面積が小さくなり、試薬担持量が少なくなってしまう。そこで、検体を供給する側の孔径を大きくし、他方の孔径を小さくする異方性構造とすることで、検体の浸み込み及び展開を早くし、必要量の試薬を担持し、かつ検体と試薬が反応する場を提供することができるため、迅速かつ正確な測定が可能となる。

【００１４】第１層の異方性の多孔質膜の具体的な構造として、平均孔径は３～１０μｍ、望ましくは３～７μｍ、より望ましくは３～５μｍであり、検体を供給する側の表面の平均孔径と第２層と接触する側の表面の平均孔径との比は２．０以上、望ましくは３．０以上、より望ましくは４．０以上である。

【００１５】第１層の膜厚は、特に限定しないが５０～２００μｍ、望ましくは７０～１８０μｍ、より望まし

くは８０～１５０μｍである。膜厚が５０μｍを下回ると、膜強度が不足してしまい、２００μｍを越えてしまうと検体の展開に時間がかかってしまうためである。

【００１６】第１層の空孔率は、特に限定しないが５０～９５%、望ましくは６０～９０%、より望ましくは７０～８８%である。空孔率が５０%を下回ると必要な量の検体を吸収展開することが出来なくなり、９５%を越えると膜強度が不足してしまうためである。

【００１７】第１層の膜材質となるポリマーとしては、ニトロセルロース、ポリビニルジフロライド、セルロースアセテート、ポリスルホン、ポリエーテルスルホン、ポリエチレンなどが使用できるが、とりわけ血糖値を測定するために使用する試薬を担持した場合には、ポリエーテルスルホンが試薬活性の経時的劣化が最も少なく好適に使用できる。

【００１８】第１層に担持される試薬としては、グルコースオキシターゼ（ＧＯＤ）様酵素、ペルオキシターゼ（ＰＯＤ）様酵素、アスコルビン酸オキシダーゼ様酵素、アルコールオキシダーゼ様酵素、及びコレステロールオキシダーゼ様酵素など酵素剤や、４－アミノアンチピリン、Ｎ－エチル－Ｎ－（２－ヒドロキシ－３－スルホプロピル）－ｍ－トルイジンなどの発色剤があげられる。なお、これらの試薬は、単独または複数で試験紙に担持される。担持の方法としては、特に限定しないが、これらの試薬をリン酸緩衝液などの緩衝液や水などに溶かして第１層に含浸させ、その後乾燥させる方法などがあげられる。

【００１９】第２層は、異方性の多孔質膜で、第１層と接触する側の表面の平均孔径が検体中の特定成分を測定する側の表面の平均孔径よりも小さいものである。第２層は、血球などの浮遊物を濾別し、試薬と反応した血漿成分などの特定成分を迅速に開放面へ展開させることが要求され、孔径を小さくすることで血球などの浮遊物を濾別・除去することができる。しかし、孔径を小さくしすぎると血漿成分などの特定成分の展開が遅くなる。また第１層と接触する側の表面の孔径を大きくし、測定する側の表面の孔径を小さくすることで展開速度を維持しつつ血球などの浮遊物を除去する方法もあるが、浮遊物の濾別・除去が特定成分を測定する側の表面近くで行われるため、血球成分の血色素が多孔質構造を通して透けて見えてしまい測定の精度に影響を及ぼしてしまう。

【００２０】そこで、第１層と接触する側の表面の孔径を小さくし、他方の孔径を大きくする異方性構造とすることで、血球などの浮遊物を濾別・除去を的確に行うとともに、血漿成分などの特定成分の測定する側の表面への展開速度、測定精度を向上させることが可能となる。

【００２１】第２層の異方性の多孔質膜の具体的な構造として、平均孔径は０．１～２μｍ、望ましくは０．３～１．６μｍ、より望ましくは０．５～１．３μｍであり、検体中の特定成分を測定する側の表面の平均孔径と第１層と接触する側の表面の平均孔径との比は１．５以上、望ましくは２．０以上、より望ましくは２．３以上である。

【００２２】第２層の膜厚は、特に限定しないが５０～２００μｍ、望ましくは７０～１８０μｍ、より望ましくは８０～１５０μｍである。膜厚が５０μｍを下回ると、膜強度が不足してしまい、２００μｍを越えてしまうと検体の展開に時間がかかってしまうためである。

【００２３】第２層の空孔率は、特に限定しないが５０～９５%、望ましくは７０～９０%、より望ましくは７５～８５%である。空孔率が５０%を下回ると必要な量の検体を吸収展開することが出来なくなり、９５%を越えると膜強度が不足してしまうためである。

【００２４】第２層の膜材質となるポリマーとしては、ニトロセルロース、ポリビニルジフロライド、セルロースアセテート、ポリスルホン、ポリエーテルスルホン、ポリエチレンなどが使用できるが、とりわけ血糖値を測定するために使用する試薬を担持した場合には、ポリエーテルスルホンが試薬活性の経時的劣化が最も少なく好適に使用できる。

【００２５】第１層及び第２層は、試薬を溶解した水溶液を含浸させて製造することや、検体の供給、展開を迅速に行うため、親水化剤を含ませる、親水性を有する材質から構成する、あるいは親水化処理を行うことが望ましい。親水化剤としては、トライトンＸ－１００などの界面活性剤、水溶性シリコン、ヒドロキシプロピルセルロース、ポリエチレングリコール、ポリプロピレングリコールなどがあげられる。親水化処理としては、プラズマ処理、グロー放電、コロナ放電、紫外線照射などの処理方法があげられる。

【００２６】第１層及び第２層の製造方法は、特に限定することなく、湿式製膜、乾式製膜、溶融製膜などにより得ることができる。具体的には、例えば、膜材質となるポリマー溶液を膜状に塗り拡げ、溶媒成分を除去し、乾燥させる方法などがあげられる。

【００２７】第１層及び第２層の積層方法も、特に限定することなく、例えば、単に重ね合わせ周囲を固定する方法、接着・融着する方法などがあげられる。

【００２８】なお、本発明の試験紙は、検体中の特定成分の測定装置に脱着可能なチップ、あるいは測定装置自体に挿入して使用させるものである。測定装置とは、血液中の糖分、コレステロールや中性脂肪などや、尿中の糖分、蛋白や潜血などを、定量的あるいは定性的に測定する装置があげられる。

【００２９】

【実施例】以下、本発明の具体的実施例について説明する。第１層及び第２層に使用する異方性の多孔質膜を、実施例１～７、比較例１～２及び比較例６～８として、以下に示す条件で製膜した。まず、表１に示す製膜原液を基材（ガラス板）上に５０ｍｌシリンジにて線状に供

給し、これをギャップ１２５μmのアプリケーターによりガラス板上に塗り拡げた。これを、表２に示す温度で調製された７０％Ｎ－メチルー２－ピロリドン（ＮＭＰ）水溶液からなる凝固浴中に浸漬し、膜材質ポリマー成分を析出させた。その後、水浴中で溶剤成分、水溶性添加剤成分を抽出除去した後、４０℃オーブン中で乾燥させて多孔質膜を得た。

【００３０】比較例３～５及び比較例９～１０については、市販されているニトロ化ポリエーテルスルホン膜およびポリエーテルスルホン膜（ともにポール・ゲルマンサイエンス社製）をそれぞれ使用した。

【００３１】

【表１】

表１　製膜原液組成

| | |
|---|---|
| 第１層製膜原液 | |
| ポリエーテルスルホン<br>（スミカエクセル７３００Ｐ、住友化学(株)製） | １０wt% |
| ポリビニルピロリドン<br>（ＢＡＳＦポビドンＫ－９０、ＢＡＳＦ製） | 5wt% |
| Ｎ－メチルー２－ピロリドン<br>（ＢＡＳＦ製） | ８５wt% |
| 第２層製膜原液 | |
| ポリエーテルスルホン<br>（スミカエクセル７３００Ｐ、住友化学(株)製） | １５　wt% |
| ポリビニルピロリドン<br>（ＢＡＳＦポビドンＫ－９０、ＢＡＳＦ製） | ７．５wt% |
| Ｎ－メチルー２－ピロリドン<br>（ＢＡＳＦ製） | ７７．５wt% |

【００３２】

【表２】

表２　製膜凝固温度

| | | 凝固浴温度(℃) |
|---|---|---|
| 第１層 | 比較例１ | ２５ |
| | 実施例１ | ３０ |
| | 実施例２ | ３５ |
| | 実施例３ | ４０ |
| | 比較例２ | ４５ |
| 第２層 | 比較例６ | ２５ |
| | 実施例４ | ３０ |
| | 実施例５ | ３５ |
| | 実施例６ | ４０ |
| | 実施例７ | ４５ |
| | 比較例７ | ５０ |
| | 比較例８ | ５５ |

【００３３】各実施例及び比較例の異方性の多孔質膜の各物性を表３及び表４に示す。各実施例及び比較例の多孔質膜の平均孔径はASTM　F316-86に従いキャピラリィ　フロー　ポロメトリィ（Capillary Flow Porometry）によって測定した。測定装置はパームポロシメーター（ＰＭＩ社製）を使用した。各実施例及び比較例の多孔質膜の表面平均孔径は走査型電子顕微鏡（JSM-840日本電子製）で撮影した画像を画像解析装置（IP-1000PC 旭化成製）により解析し、視野内の孔の孔径を面積換算で円相当径として算出し、相加平均を表面平均孔径とした。したがって、多孔質膜の平均孔径と、表面平均孔径は必ずしも相関関係が成り立つわけではない。膜厚はマイクロメーター（ミツトヨ精機製）にて測定した。空孔率は重量法にて測定した。多孔質膜各部の条件は、次の通りである。

【００３４】［第１の層］

材質：ポリエーテルスルホン

膜厚：１３０±５μｍ

空孔率：８２±３％

コートした試薬：ＧＯＤ、ＰＯＤおよび４－アミノアンチピリン、Ｎ－エチルー－Ｎ－（２－ヒドロキシー３－スルホプロピル）－ｍ－トルイジン（ＴＯＯＳ）、トライトンＸ－１００

【００３５】［第２の層］

材質：ポリエーテルスルホン

膜厚：１００±５μｍ

空孔率：８４±３％

コートした試薬：トライトンＸ－１００

【００３６】

【表３】

表３

| 第１層 | 平均孔径（μｍ） | 表面平均孔径（μｍ） | | 孔径大/小比 |
|---|---|---|---|---|
| | | 開放面（孔径小面） | 基材側面（孔径大面） | |
| 比較例１　異方性膜 | １．０ | ０．２ | ２．０ | ８．９ |
| 実施例１　異方性膜 | ３．１ | ０．２ | ２．４ | １０．６ |
| 実施例２　異方性膜 | ５．０ | ０．２ | ４．７ | ２１．５ |
| 実施例３　異方性膜 | １０．０ | ０．３ | ５．５ | ２１．１ |
| 比較例２　異方性膜 | １５．０ | ０．３ | ６．３ | ２０．４ |
| 比較例３　等方性膜 | １．０ | ０．８ | １．０ | １．２ |
| 比較例４　等方性膜 | ２．６ | ２．４ | ２．５ | １．１ |
| 比較例５　等方性膜 | ５．０ | ４．６ | ４．８ | １．０ |

【００３７】

【表４】

表４

| 第２層 | 平均孔径（μｍ） | 表面平均孔径（μｍ） | | 孔径大/小比 |
|---|---|---|---|---|
| | | 開放面（孔径小面） | 基材側面（孔径大面） | |
| 比較例６　異方性膜 | ０．０５ | ０．１ | ０．２ | ３．６ |
| 実施例４　異方性膜 | ０．１０ | ０．１ | ０．３ | ２．４ |
| 実施例５　異方性膜 | ０．５ | ０．２ | １．４ | ７．８ |
| 実施例６　異方性膜 | １．０ | ０．２ | ２．０ | ８．９ |
| 実施例７　異方性膜 | ２．０ | ０．２ | １．９ | ８．５ |
| 比較例７　異方性膜 | ３．１ | ０．２ | ２．４ | １０．６ |
| 比較例８　異方性膜 | ５．０ | ０．２ | ４．７ | ２１．５ |
| 比較例９　等方性膜 | ０．２２ | ０．５ | ０．６ | １．２ |
| 比較例10　等方性膜 | ０．４５ | １．０ | １．３ | １．３ |

【００３８】（試験例１）実施例及び比較例の第１層の多孔質膜を用い、次の試験を行った。評価する多孔質膜表面の反射吸光度が測定できるように分光光度計（ＵＶ－２４００（ＰＣ）Ｓ　島津製作所社製）のサンプルホルダーに固定し、ヒト血液を測定面反対側へマイクロピペット（エッペンドルフ社製）で５μｌ添加し、反対面の反射吸光度の時間変化を測定した。異方性膜については、血液の添加を孔径の大きい面と小さい面の二通りについて行った。１秒間の反射率の変化割合が、最終変化量の１％を超えた時から、１秒間の反射率の変化割合が最終変化量の１％を下回ったときまでの時間をしみ出し時間Δｔとした。測定条件は下記のとおりである。結果を表５に示す。

【００３９】測定条件（時間変化）

測光値：反射率

波長：６１０ｎｍ

スリット幅：２．０ｎｍ

タイミングモード：オート

測定時間：９０秒

サンプリングピッチ：０．１ｓｅｃ

セル数：１

データ数：９０１

【００４０】

【表５】

表５

| 第１層 | 試験例１　しみ出し時間Δｔ | | 試験例２ |
|---|---|---|---|
| | 孔径大面から添加（sec） | 孔径小面から添加（sec） | 試薬担持量（mg/cm2） |
| 比較例１　異方性膜 | ８．３ | ５．７ | １．１ |
| 実施例１　異方性膜 | ２．６ | ４．６ | １．０ |
| 実施例２　異方性膜 | １．８ | ３．５ | １．０ |
| 実施例３　異方性膜 | １．４ | ３．１ | ０．９ |
| 比較例２　異方性膜 | ０．８ | ２．６ | ０．７ |
| 比較例３　等方性膜 | １０．９ | － | ２．２ |
| 比較例４　等方性膜 | ４．４ | － | １．５ |
| 比較例５　等方性膜 | ２．９ | － | ０．６ |

【００４１】（試験例２）実施例及び比較例の第１層の多孔質膜について、次の通り試薬担持量の測定を行った。多孔質膜の試薬コート前の重量を精秤し、試薬コート後の重量から差し引くことで、試薬担持量を算出した。結果を表５に示す。

【００４２】（試験例３）実施例及び比較例の第２層の多孔質膜を用い、次の試験を行った。評価する多孔質膜表面の反射吸光度が測定できるように分光光度計（ＵＶ

－２４００（ＰＣ）Ｓ　島津製作所社製）のサンプルホルダーに固定し、ヒト血液を測定面反対側へマイクロピペット（エッペンドルフ社製）で５μｌ添加し、試験例１と同じしみ出し時間Δｔの測定と、反対面の反射吸光度の反射吸光スペクトルを測定した。しみ出し時間Δｔの測定条件は試験例１と同じである。血漿を添加した場合のスペクトルと比較し血色素の影響の有無を判定した。異方性膜については、血液の添加を孔径の大きい面と小さい面の二通りについて行った。結果を表６に示す。

【００４３】測定条件（反射吸光スペクトル）
測光値：反射率
波長範囲（ｎｍ）：開始７００，終了５００
スキャン速度：中速
スリット幅：２．０ｎｍ
サンプリングピッチ：１．０ｎｍ

【００４４】
【表６】

表６

| 第２層 | 試験例３ | | | |
|---|---|---|---|---|
| | しみ出し時間Δｔ | | 血色素の影響 | |
| | 孔径大面から添加（sec） | 孔径小面から添加（sec） | 孔径大面 | 孔径小面 |
| 比較例６　異方性膜 | １０．２ | ３７．７ | なし | なし |
| 実施例４　異方性膜 | ７．２ | ３５．２ | なし | なし |
| 実施例５　異方性膜 | ６．８ | １５．９ | なし | なし |
| 実施例６　異方性膜 | ５．７ | ８．３ | あり | なし |
| 実施例７　異方性膜 | ５．２ | ５．４ | あり | なし |
| 比較例７　異方性膜 | ４．６ | ２．６ | あり | なし |
| 比較例８　異方性膜 | ３．５ | １．８ | あり | あり |
| 比較例９　等方性膜 | ８．７ | － | あり | － |
| 比較例10　等方性膜 | ８．１ | － | あり | － |

【００４５】（試験例４）上記の第１層と第２層の各多孔質膜を積層する次の実験を行った。分光光度計（ＵＶ－２４００（ＰＣ）Ｓ　島津製作所社製）のサンプルホルダーに、上記の第１層と第２層の各多孔質膜が密着するように重ねて固定し、ヒト血液を入り口側へマイクロピペット（エッペンドルフ社製）で５μｌ添加し、反対面の反射吸光度の時間変化および反射吸光スペクトルを測定した。

【００４６】この時、血液を添加する側に第１層の多孔質膜、測定する側に第２層の多孔質膜を固定した。また、各多孔質膜の固定する向きについては、等方性膜以外は、第１層は孔径の大きい面を血液を添加する側に、第２層は孔径の大きい面を測定する側にして実験を行った。１秒間の反射率の変化割合が、最終変化量の１％を超えた時から、１秒間の反射率の変化割合が最終変化量の１％を下回ったときまでの時間をしみ出し時間Δｔとした。

【００４７】また、血漿を添加した場合のスペクトルと比較し血色素の影響の有無を判定した。各測定条件は試験例１および試験例３と同じである。これらの結果を表７に示す。

【００４８】
【表７】

表７

| | | | 試験例４ | |
|---|---|---|---|---|
| | 第１層 | 第２層 | しみ出し時間Δｔ（sec） | 血色素の影響 |
| 比較例11 | 比較例１ | 比較例６ | １５．３ | なし |
| 比較例12 | 比較例１ | 実施例４ | １５．３ | なし |
| 比較例13 | 実施例１ | 比較例６ | １１．９ | なし |
| 実施例８ | 実施例１ | 実施例４ | ９．１ | なし |
| 実施例９ | 実施例１ | 実施例７ | ７．４ | なし |
| 実施例10 | 実施例２ | 実施例５ | ７．７ | なし |
| 実施例11 | 実施例２ | 実施例６ | ６．５ | なし |
| 実施例12 | 実施例３ | 実施例４ | ８．６ | なし |
| 実施例13 | 実施例３ | 実施例７ | ６．２ | なし |
| 比較例14 | 実施例３ | 比較例７ | ６．０ | あり |
| 比較例15 | 比較例３ | 比較例９ | １８．１ | なし |
| 比較例16 | 比較例５ | 比較例10 | ９．８ | あり |

【００４９】
【発明の効果】本発明の試験紙は、検体の展開速度が速く、展開に要する時間を短くすることができるとともに、測色に際し、十分な試薬担持量が確保でき、浮遊物の影響を排し、より高精度の測定を行うことができる。すなわち、検体の展開に要する時間を飛躍的に短縮することができ、かつ非常に測定精度が高い、検体中の特定成分測定用試験紙を提供することができる。

【００５０】特に、本発明は血液中の糖分を測定する血糖試験紙として有効であり、血液の展開速度が速く、展開に要する時間を短くすることができるとともに、血糖値の測色に際し、十分な酵素剤や発色剤などの試薬担持

量が確保でき、赤血球等の浮遊物の影響を排し、より高精度の測定を行うことができる。

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